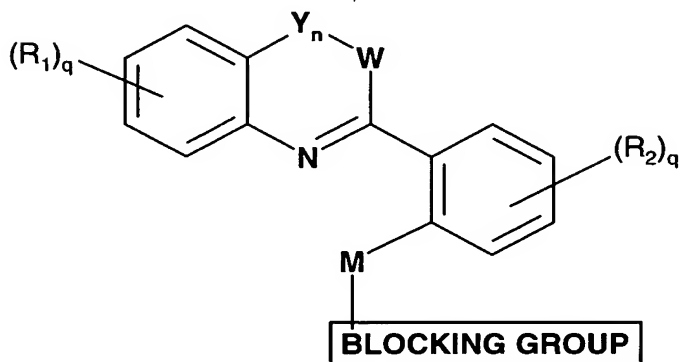


Listing of Claims:

This listing of claims replaces all prior versions, and listings, of claims in the captioned application.

- (Original) An enzyme substrate of the formula (I):



(I)

and biologically acceptable salts, and pro-reporter molecules thereof;

wherein

Y is >C=O , >C=CH_2 , >C=R_1 , and

n is 1 or 0;

W is =CH-, -S-, -O-, or -N(R₃)-;

M is oxygen, nitrogen or sulfur;

R₁ and **R₂** are, each independently, hydrogen, halogen, nitro, azido, mercapto, sulfeno, sulfino, sulfo, cyano, amino, R₄-, R₄O-, R₄C(=Z)-, R₄X-C(=Z)-,

R₄-C(=Z)-X-, R₄X-C(=Z)-Q-, R₄S-, R₄-S(=O)-, R₄-S(=O)-O-, R₄-S(=O)₂-O-, R₄O-S-, R₄O-S(=O)-, R₄O-S(=O)₂-, R₄R₅N-S(=O)-, R₄R₅N-S(=O)₂-, R₄R₅N-, [R₄-C(=Z)][R₅]N-, [R₄-C(=Z)][R₅-C(=X)]N-, R₄R₅N-C(=Z)-, R₄R₅N-C(=Z)-X-, [R₄R₅N-C(=Z)][R₆]N-, [R₄R₅N-C(=Z)][R₆-C(=X)]N-, [R₄-S(=O)][R₅]N-, [R₄-S(=O)₂][R₅]N-, (R₄X)(R₅Q)P(=Z)-, (R₄R₅N)(R₆X)P(=Z)-, (R₄R₅N)(R₆R₇N)P(=Z)-, (R₄X)(R₅Q)P(=Z)-O-, (R₄R₅N)(R₆X)P(=Z)-O-, (R₄R₅N)(R₆R₇N)P(=Z)-O-, (R₄X)(R₅Q)P(=Z)-S-, (R₄R₅N)(R₆X)P(=Z)-S-, (R₄R₅N)(R₆R₇N)P(=Z)-S-, [(R₄X)(R₅Q)P(=Z)][R₆]N-, [(R₄R₅N)(R₆X)P(=Z)][R₇]N-, [(R₄R₅N)(R₆R₇N)P(=Z)][R₈]N-, (R₄)(R₅X)P(=Z)-O-, (R₄)(R₅R₆N)P(=Z)-O-,

$(R_4)(R_5X)P(=Z)-S-$, $(R_4)(R_5R_6N)P(=Z)-S-$, $[(R_4)(R_5X)P(=Z)][R_6]N-$,
 $[(R_4)(R_5R_6N)P(=Z)][R_7]N-$;

wherein **X**, **Z** and **Q** are each independently oxygen or sulfur;

R₃ is **R₄**, **R₄-C(=Z)-**, **R₄X-C(=Z)-**, **R₄R₅N-C(=Z)-**, **R₄O-S(=O)-**, **R₄O-S(=O)₂-**,
R₄R₅N-S(=O)-, **R₄R₅N-S(=O)₂-**, **(R₄X)(R₅Q)P(=Z)-**, **(R₄R₅N)(R₆X)P(=Z)-**,
(R₄R₅N)(R₆R₇N)P(=Z)-;

wherein **R₄**, **R₅**, **R₆**, **R₇** and **R₈** are, each independently, hydrogen, C₁₋₈alkyl,
C₂₋₈alkenyl, C₂₋₈alkynyl, C₃₋₇cycloalkyl, aryl, Het¹, Het²;

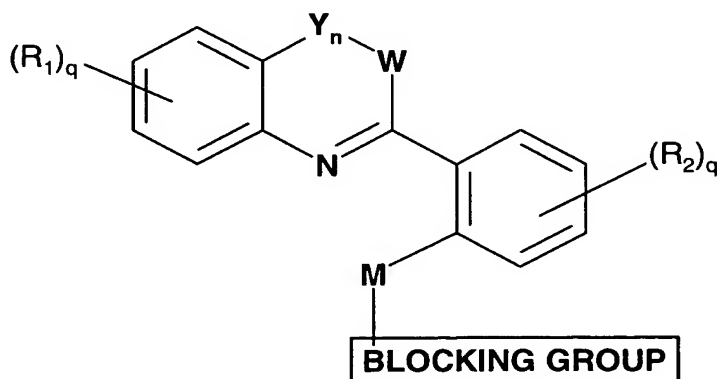
each **q** is independently 0, 1, 2, 3, or 4;

wherein any C₁₋₈alkyl, C₂₋₈alkenyl, C₂₋₈alkynyl or amino group, may be further mono,
di-, or tri- substituted (if the valency allows it) with C₁₋₄alkoxy, C₁₋₄alkylcarbonyl,
C₁₋₄alkoxycarbonyl, C₁₋₄alkylthio, C₁₋₄alkylsulfenyl, C₁₋₄alkylsulfinyl,
C₁₋₄alkylsulfonyl, C₁₋₄alkylamino, halogen, nitro, azido, mercapto, sulfeno, sulfino,
sulfo, cyano, amino, aryl, Het¹ or Het² substituents;

wherein the **BLOCKING GROUP** is a mono- or polysaccharide derivate, phosphate
derivate, or sulfate derivate;

with the proviso that at least one **R₁**, **R₂** and **R₃** is a moiety with at least 4 carbons.

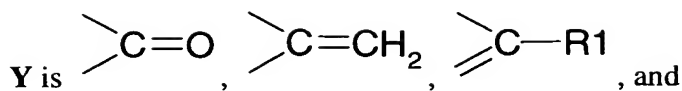
2. (Original) An enzyme substrate of the formula (I):



(I)

and biologically acceptable salts and pro-reporter molecules thereof;

wherein



n is 1 or 0;

W is =CH-, -S-, -O-, or -N(R₃)-;

M is -O-, -N(R₃)-, or -S-;

each **R₁** and each **R₂** present in formula (I) are, independently, hydrogen, halogen, nitro, azido, mercapto, sulfeno, sulfinio, sulfo, cyano, amino, R₄-, R₄O-, R₄-C(=Z)-, R₄X-C(=Z)-, R₄-C(=Z)-X-, R₄X-C(=Z)-Q-, R₄S-, R₄-S(=O)-, R₄-S(=O)₂-, R₄-S(=O)-O-, R₄-S(=O)₂-O-, R₄O-S-, R₄O-S(=O)-, R₄O-S(=O)₂-, R₄R₅N-S(=O)-, R₄R₅N-S(=O)₂-, R₄R₅N-, [R₄-C(=Z)][R₅]N-, [R₄-C(=Z)][R₅-C(=X)]N-, [R₄X-C(=Z)][R₅]N-, [R₄X-C(=Z)][R₅-C(=Q)]N-, R₄R₅N-C(=Z)-, R₄R₅N-C(=Z)-X-, [R₄R₅N-C(=Z)][R₆]N-, [R₄R₅N-C(=Z)][R₆C(=X)]N-, [R₄-S(=O)][R₅]N-, [R₄-S(=O)₂][R₅]N-, (R₄X)(R₅Q)P(=Z)-, (R₄R₅N)(R₆X)P(=Z)-, (R₄R₅N)(R₆R₇N)P(=Z)-, (R₄X)(R₅Q)P(=Z)-O-, (R₄R₅N)(R₆X)P(=Z)-O-, (R₄R₅N)(R₆R₇N)P(=Z)-O-, (R₄X)(R₅Q)P(=Z)-S-, (R₄R₅N)(R₆X)P(=Z)-S-, (R₄R₅N)(R₆R₇N)P(=Z)-S-, [(R₄X)(R₅Q)P(=Z)][R₆]N-, [(R₄R₅N)(R₆X)P(=Z)][R₇]N-, [(R₄R₅N)(R₆R₇N)P(=Z)][R₈]N-, (R₄)(R₅X)P(=Z)-O-, (R₄)(R₅R₆N)P(=Z)-O-, (R₄)(R₅X)P(=Z)-S-, (R₄)(R₅R₆N)P(=Z)-S-, [(R₄)(R₅X)P(=Z)][R₆]N-, [(R₄)(R₅R₆N)P(=Z)][R₇]N-;

wherein **X**, **Z** and **Q** are each, independently, O or S;

R₃ is R₄, R₄-C(=Z)-, R₄X-C(=Z)-, R₄R₅N-C(=Z)-, R₄O-S(=O)-, R₄O-S(=O)₂-, R₄R₅N-S(=O)-, R₄R₅N-S(=O)₂-, (R₄X)(R₅Q)P(=Z)-, (R₄R₅N)(R₆X)P(=Z)-, (R₄R₅N)(R₆R₇N)P(=Z)-;

wherein **R₄**, **R₅**, **R₆**, **R₇** and **R₈** are each, independently, hydrogen, C₁₋₈alkyl, C₂₋₈alkenyl, C₂₋₈alkynyl, C₃₋₇cycloalkyl, aryl, Het¹, Het²;

each **q** present in formula (I) is, independently, 0, 1, 2, 3, or 4;

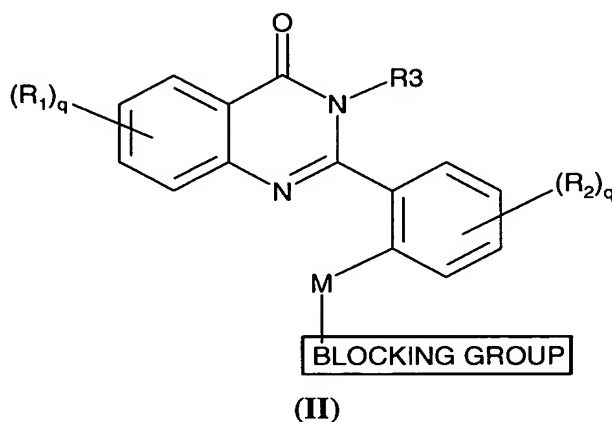
wherein any C₁₋₈alkyl, C₂₋₈alkenyl, C₂₋₈alkynyl or amino group, may be further mono-, di-, or tri- substituted (if the valency allows it) with C₁₋₄alkoxy, C₁₋₄alkylcarbonyl, C₁₋₄alkoxycarbonyl, C₁₋₄alkylthio, C₁₋₄alkylsulfenyl, C₁₋₄alkylsulfinyl, C₁₋₄alkylsulfonyl, C₁₋₄alkylamino, halogen, nitro, azido, mercapto, sulfeno, sulfinio, sulfo, cyano, amino, aryl, Het¹ or Het² substituents;

wherein the **BLOCKING GROUP** is a mono- or polysaccharide derivate, phosphate derivate, sulfate derivate, carboxylic acid derivate, or oligopeptide derivate;

with the proviso that at least one of **R₁**, **R₂** and **R₃** is C₄₋₈alkyl, C₄₋₈alkenyl, or C₄₋₈alkynyl.

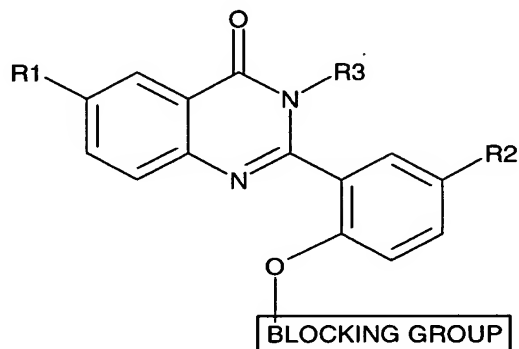
3. (Currently Amended) A substrate according to ~~any one of claims claim 1 to 2~~, wherein at least one of **R₁**, **R₂** and **R₃** is independently chosen from the group consisting of straight and branched butyl, pentyl, hexyl, heptyl, octyl.

4. (Currently Amended) A substrate according to ~~any one of claims claim 1 to 3~~, wherein W is -N(R₃)-, Y is -C(=O)-, and n is 1 and having the formula (II)



wherein **M**, **R₁**, **R₂**, **R₃**, **q** and the **BLOCKING GROUP** are as defined as in ~~any one of claims claim 1 to 3~~.

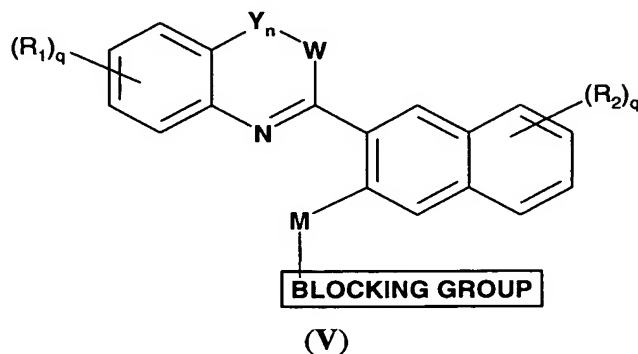
5. (Currently Amended) A substrate according to ~~any one of claims claim 1 to 4~~, having the formula (III)



(III)

wherein **R₁**, **R₂**, **R₃**, and the **BLOCKING GROUP** are as defined as in ~~any one of~~ claims claim 1 to 3.

6. (Original) An enzyme substrate of the formula (V):



and biologically acceptable salts, and pro-reporter molecules thereof;

wherein

Y is >C=O , >C=CH_2 , >C=R1 , and

n is 1 or 0;

W is =CH-, -S-, -O-, or -N(R₃)-;

M is -O-, -N(R₃)-, or -S-;

each **R₁** and each **R₂** present in formula (V) are, independently, hydrogen, halogen, nitro, azido, mercapto, sulfeno, sulfino, sulfo, cyano, amino, **R₄**-, **R₄O**-, **R₄-C(=Z)**-, **R₄X-C(=Z)**-, **R₄-C(=Z)-X**-, **R₄X-C(=Z)-Q**-, **R₄S**-, **R₄-S(=O)**-, **R₄-S(=O)₂**-, **R₄-S(=O)-O**-, **R₄-S(=O)₂-O**-, **R₄O-S**-, **R₄O-S(=O)**-, **R₄O-S(=O)₂**-, **R₄R₅N-S(=O)**-, **R₄R₅N-S(=O)₂**-, **R₄R₅N**-, **[R₄-C(=Z)][R₅N]**-, **[R₄-C(=Z)][R₅-C(=X)]N**-, **[R₄X-C(=Z)][R₅N]**-, **[R₄X-C(=Z)][R₅-C(=Q)]N**-, **R₄R₅N-C(=Z)**-, **R₄R₅N-C(=Z)-X**-, **[R₄R₅N-C(=Z)][R₆N]**-, **[R₄R₅N-C(=Z)][R₆C(=X)]N**-, **[R₄-S(=O)][R₅N]**-, **[R₄-S(=O)₂][R₅N]**-, **(R₄X)(R₅Q)P(=Z)**-, **(R₄R₅N)(R₆X)P(=Z)**-, **(R₄R₅N)(R₆R₇N)P(=Z)**-, **(R₄X)(R₅Q)P(=Z)-O**-, **(R₄R₅N)(R₆X)P(=Z)-O**-, **(R₄R₅N)(R₆R₇N)P(=Z)-O**-, **(R₄X)(R₅Q)P(=Z)-S**-, **(R₄R₅N)(R₆X)P(=Z)-S**-, **(R₄R₅N)(R₆R₇N)P(=Z)-S**-, **[(R₄X)(R₅Q)P(=Z)][R₆N]**-, **[(R₄R₅N)(R₆X)P(=Z)][R₇N]**-, **[(R₄R₅N)(R₆R₇N)P(=Z)][R₈N]**-, **(R₄)(R₅X)P(=Z)-O**-, **(R₄)(R₅R₆N)P(=Z)-O**-, **(R₄)(R₅X)P(=Z)-S**-, **(R₄)(R₅R₆N)P(=Z)-S**-, **[(R₄)(R₅X)P(=Z)][R₆N]**-, **[(R₄)(R₅R₆N)P(=Z)][R₇N]**-;

wherein the **R₂** substituent can replace one or more hydrogens on any carbon atom of the naphthyl group, such as carbon atoms C1, C4, C5, C6, C7, and C8, provided that the carbon's valency is not exceeded;
wherein **X, Z and Q** are each, independently, O or S;

R₃ is **R₄**, **R₄-C(=Z)-**, **R₄X-C(=Z)-**, **R₄R₅N-C(=Z)-**, **R₄O-S(=O)-**, **R₄O-S(=O)₂-**, **R₄R₅N-S(=O)-**, **R₄R₅N-S(=O)₂-**, **(R₄X)(R₅Q)P(=Z)-**, **(R₄R₅N)(R₆X)P(=Z)-**, **(R₄R₅N)(R₆R₇N)P(=Z)-**;

wherein **R₄**, **R₅**, **R₆**, **R₇** and **R₈** are each, independently, hydrogen, C₁₋₈alkyl, C₂₋₈alkenyl, C₂₋₈alkynyl, C₃₋₇cycloalkyl, aryl, Het¹, Het²;

each **q** present in formula (V) is, independently, 0, 1, 2, 3, or 4;

wherein any C₁₋₈alkyl, C₂₋₈alkenyl, C₂₋₈alkynyl or amino group, may be further mono, di-, or tri- substituted (if the valency allows it) with C₁₋₄alkoxy, C₁₋₄alkylcarbonyl, C₁₋₄alkoxycarbonyl, C₁₋₄alkylthio, C₁₋₄alkylsulfenyl, C₁₋₄alkylsulfinyl, C₁₋₄alkylsulfonyl, C₁₋₄alkylamino, halogen, nitro, azido, mercapto, sulfeno, sulfinio, sulfo, cyano, amino, aryl, Het¹ or Het² substituents;

wherein the **BLOCKING GROUP** is a mono- or polysaccharide derivate, phosphate derivate, sulfate derivate, carboxylic acid derivate, or oligopeptide derivate;

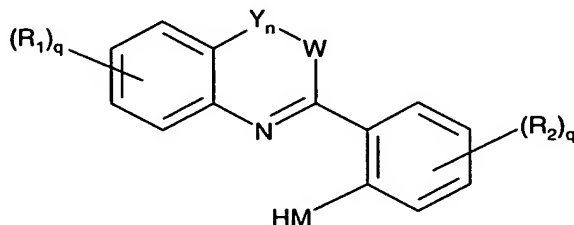
with the proviso that at least one of **R₁**, **R₂** and **R₃** is C₄₋₈alkyl, C₄₋₈alkenyl, or C₄₋₈alkynyl.

7. (Currently Amended) Use of a substrate according to ~~any one of claims~~ claim 1 to 6, for permeation through the membrane of a biological cell.

8. (Currently Amended) Method for preparing a substrate according to ~~any one of claims~~ claim 1 to 6 comprising the steps of:

- synthesizing a blocking group, whereby said blocking group may be optionally protected;
- synthesizing a substituted fluorophore;
- coupling the optionally protected blocking group to said substituted fluorophore;
- optionally deprotecting said blocking group; and
- purifying the resulting substituted substrate.

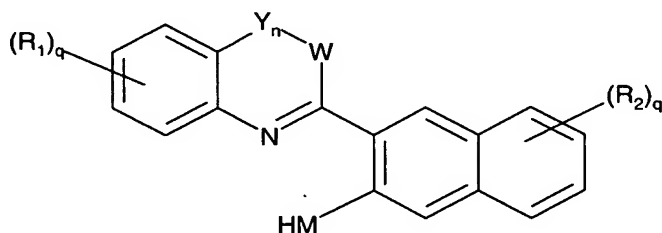
9. (Currently Amended) A fluorescent precipitate obtainable by cleavage of the **BLOCKING GROUP** moiety from the substrate of formula (I), ~~(II), and (III)~~ of ~~any one of the claims claim 1 to 5~~, having the formula (IV)



(IV)

wherein Y , n , W , M , R_1 , R_2 and q are as defined as in ~~any one of claims claim 1 to 5~~.

10. (Original) A fluorescent precipitate obtainable by cleavage of the **BLOCKING GROUP** moiety from the substrate of formula (V) of claim 6, having the formula (VI):

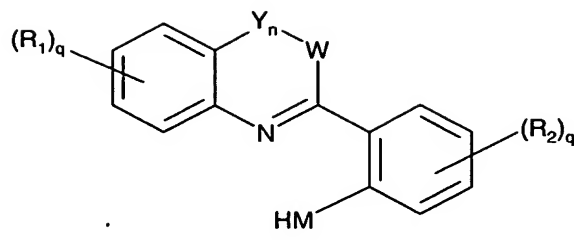


(VI)

wherein Y , n , W , M , R_1 , R_2 and q are as defined as in claim 6.

11. (Currently Amended) Method for detecting the activity of an enzyme comprising the steps of:

- contacting a sample containing said enzyme with a substrate according to ~~any one of claims claim 1 to 6~~;
applying conditions suitable to allow formation of a fluorescent precipitate, said fluorescent precipitate comprising a fluorescent precipitate obtainable by cleavage of the **BLOCKING GROUP** moiety from the substrate of formula (I) of claim 1, having the formula (IV)



(IV)

wherein Y, n, W, M, R₁, R₂ and q are as defined as in claim 1 according to any one of claims 9 to 10; and

- quantitatively or qualitatively analyzing said fluorescent precipitate.

12. (Original) Method according to claim 11 wherein analyzing said fluorescent precipitate comprises the steps of:

- exposing the fluorescent precipitate to a light source capable of producing light at a wavelength of absorption of the fluorescent precipitate; and
- detecting the resultant fluorescence of the precipitate.